respectively), that persisted for sHA and uCTX-II after adjustment for age, gender and BMI (stand betas 0.113, P=0.003 and 0.129, P=0.000, respectively). Only cartilage marker combinations showed stronger associations with baseline summed K&L grades than individual biomarkers (stand beta 0.150, P=0.000 for uCTX-II+sCOMP+PIIANP+CS846 combination, after adjustment).
Associations with AUC of the summed K&L score were statistically significant for uCTX-II, sCOMP, sPIIANP, and sCS846 (stand betas 0.089-0.133, P≤0.018) and persisted after adjustment for age, gender, BMI, and baseline summed K&L score for sCOMP only (stand beta 0.105, P=0.005). Stronger associations were observed for cartilage and synovial combinations (stand beta 0.128, P=0.001 for uCTXII+sCOMP+sPIIANP+sCS846, and stand beta 0.110, P=0.003 for sCOMP+sPIIINP).
**Conclusions:** Associations with burden and progression of (very) early knee and hip OA were observed for cartilage and synovial biomarkers. The low grade of the associations may be due to limitations of biomarkers as well as radiography in reflecting disease in OA, especially in early-stage disease. Associations may be stronger at 10-year follow-up and/or when using more sensitive measures of individual radiographic OA features (e.g. osteophytes, joint space narrowing, etc). Nevertheless, together with literature data these results indicate that the search for biomarkers for (very) early OA should be primarily aimed at (combinations of) cartilage and synovial metabolism.
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## OSTEOARTHRITIS BIOMARKERS IN HUMAN SERUM BY CHEMICAL DEPLETION COUPLED TO TWO DIMENSIONAL DIFFERENCE IN GEL ELECTROPHORESIS

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**Purpose:** The aim of this study is to search for osteoarthritis (OA) biomarkers in human serum using a novel chemical sequential depletion method combined with two dimensional difference in-gel electrophoresis (2D-DIGE) analyses.
**Methods:** The serum samples were obtained from 20 OA pacientes and 20 non-symptomatic controls. Samples were grouped into four pools of 10 samples each. The pools were subjected to a chemical sequential depletion protocol involving two precipitation steps, first with DTT and then with ACN, to reduce the high dynamic range of the proteins. Then, the proteomics comparison between OA and control sera was performed across four DIGE gels. Samples from control and OA pools were labeled with Cy3 or Cy5 dyes, mixed by pairs and co-resolved by two-dimensional gel electrophoresis using a pool of all samples labeled with Cy2 as internal standard. The gels were imaged using a DIGE Imager scanner, and quantitative image analysis was performed using Same Spots software. For protein identification, gel spots were digested by in-gel enzymatic digestion accelerated with ultrasounds. Then, peptides were analyzed by mass spectrometry (MALDI-TOF/TOF) and identified using Mascot with SwissProt knowledgebase. Verification of the alteration of SAA and HPT was performed by immunoblotting.
**Results:** We report the combination of a novel chemical sequential depletion method combined with 2D-DIGE for the search of OA biomarkers. An average of 210 protein spots were detected and matched on the gel images. The differential analysis resulted in 42 spots significantly and reproducibly altered between OA and control samples, 29 increased and 17 decreased. These 45 spots corresponded to 14 different proteins, many of them related with lipid transport, immune response or protein binding. We identified a spot corresponding to a serum amyloid A protein form (SAA), which was decreased in OA sera when compared to control. SAA is a major acute phase reactant implicated in the pathogenesis of rheumatoid arthritis, however, the role of SAA in OA has been poorly investigated. We also detected two spots corresponding to haptoglobin precursor (HPT) that exhibited an opposite alteration in OA sera when compared to control. HPT precursor is an N-linked glycoprotein known to be cleaved into two chains, alpha and beta, which combine with free plasma hemoglobin and contribute to the maintenance of cellular iron homeostasis. Interestingly, HPT beta chain was increased in OA sera whilst HPT alpha chain was decreased in OA sera when compared to control. Western blot analyses were performed to confirm these results. The detection of modulated SAA and HPT protein forms in this work highlight the usefulness of 2D gel-based approaches for characterizing disease-specific patterns of protein modifications, which are impossible to obtain from peptide/MS-based proteomic strategies.
**Conclusions:** We were able to identify 15 protein forms altered in the disease, corresponding to 14 different proteins. The chemical sequential depletion coupled to a DIGE-based quantitative proteomic analysis is useful for protein biomarker discovery. We provide a list of potential OA biomarker candidates that might be the subject of further validation studies.

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## A DESCRIPTIVE STUDY OF SYNOVIAL FLUID CHANGES IN CYTOKINE, CHEMOKINE AND GROWTH FACTOR LEVELS BETWEEN OSTEOARTHRITIS PATIENTS AND HEALTHY DONORS.

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**Purpose:** Many studies have shown differences between the healthy and osteoarthritic (OA) joint environment. These studies are mainly based on a limited set of cytokines, chemokines or growth factors. A comprehensive profile of these kind of mediators for OA patients could potentially contribute to the search for new biomarkers or biomarker profiles. In the current study, we analysed 49 soluble mediators in synovial fluid (SF) in order to examine differences in the healthy and OA joint environment profile.
**Methods:** SF was obtained from donors with macroscopically healthy knee joints (post-mortem material; n=16) and from OA patients (total knee arthroplasy for OA; n=18). All samples were analyzed for a set of 49 different cytokines, chemokines and growth factors using multiplex ELISA assays. A non-parametric Mann-Whitney U test was used to determine significant differences. Significance was set at p<0.001.
**Results:** The majority of the soluble mediators could be detected in both healthy and OA SF. IL-6, IP-10, MDC, PDGF-AA and RANTES were significantly increased in OA compared to healthy SF (Table 1.; p<0.001). Leptin, IL-13, MIP1-α, MIP1-β, sCD-40L were also increased in OA compared to healthy SF, however less significant (p<0.05). In contrast, eotaxin and G-CSF were decreased in OA synovial fluid (p<0.05). The levels of anabolic factors such as FGF-2 and TGF-ß were not different between healthy and OA SF samples.

**Table 1**
Significantly different concentrations of cytokines, chemokines and growth factors in healthy and osteoarthritic synovial fluid (average ± sd; pg/ml). #p<0.001 and *p<0.05

| | Healthy (average ± sd; pg/ml) | OA (average ± sd; pg/ml) | P-value |
|---|---|---|---|
| Eotaxin | 22.4 ± 21.4 | 8.0 ± 18.8 | *0.02 |
| G-CSF | 90.1 ± 95.4 | 22.4 ± 15.0 | *0.03 |
| IL-6 | 57.0 ± 112.2 | 186.1 ± 224.1 | #0.001 |
| IP-10 | 521.5 ± 561.2 | 1073.6 ± 999.0 | #0.001 |
| MDC | 55.6 ± 24.4 | 210.1 ± 87.4 | #0.001 |
| MIP-1a | 4.5 ± 1.2 | 16.5 ± 28.2 | *0.05 |
| MIP-1ß | 12.7 ± 10.6 | 35.0 ± 44.7 | *0.04 |
| PDGF-AA | 1.5 ± 3.0 | 122.6 ± 89.3 | #0.001 |
| RANTES | 31.7 ± 48.3 | 762.9 ± 871.9 | #0.001 |
| sCD40L | 1.0 ± 2.3 | 29.2 ± 42.3 | *0.005 |
| Leptin | 837.3 ± 1115 | 3008.1 ± 3770 | *0.01 |
| IL-13 | nd | 18.1 ± 39.5 | *0.01 |